New post on **Stranded By Choice**

CAST vs YCA

by Mari West

First things first – I apologize for the somewhat technical nature of the post that you are about to read. I did my best to write it in such a way that anyone interested would be able to understand it, but, of course, I may have done a terrible job. If anything (or everything) is difficult to grasp, feel free to shoot questions my way. As usual, I threw in some photos, to help break up the monotony, and so that even if the written content is sub-par, at least you have something pretty to look at.

The Yellow Crazy Ant (*Anoplolepis gracilipes;* YCA), so named because of the erratic behavior it displays when disturbed, is our number one enemy here on Johnston. These little yellow ants may seem harmless at first glance, but they're a serious threat to the wildlife that the Johnston Atoll National Wildlife Refuge was created to protect.

YCA were first discovered on Johnston in 2010, a few years after the military left, by some Fish and Wildlife folks stopping by to check on the state of the island. It is believed that they were brought here by one of the many trespassing "yachties" who had stopped by while Johnston remained unoccupied. The YCA were restricted to the center of the island (mainly what is considered to be the original island, before the Air Force expanded it through coral dredging), but within its confines, the infestation was rampant. Apparently, just walking on concrete areas left behind orange footprints made up of smashed YCA.

YCA have the ability to form super-colonies – individual nests are capable of containing hundreds of queens and members of one nest do not discriminate against members of another. They can reproduce at an astonishing rate and move freely between nests, giving them a competitive edge over other species. This has allowed them to become such a successful and widespread invasive in the tropics, that it is unknown where they first originated. Unfortunate for tropicbirds, which like to nest in shady spots under trees and bushes, YCA also seek out these areas to build their nests, and they consider the presence of tropicbirds a threat. In defense, YCA will mob nesting tropicbirds, spraying formic acid out of their abdomens. If this formic acid gets into the tropicbirds' eyes, it can cause their nictitating membranes to swell which, in serious cases, can lead to blindness and eventual death. The FWS biologists realized that this was a major problem, decided

that something had to be done, and CAST was born!

A red-tailed tropicbird that we found blinded by formic acid after being attacked by YCA. Also notice the slight corrosion on the bird's beak.

It took some trial and error and a few CAST crews, but CAST III, which our own Kevin was a part of, finally saw some success (not without help from CAST II, who did some incredibly important experimental work). Their baiting efforts led to a 99% reduction in detectable YCA! However, it soon became clear that the poison being deployed was only reaching those ants that were actively foraging. Underground, hundreds of queens were still reproducing, with thousands of workers attending to them. The baiting methodology that they used was effective, but only at knocking back the YCA population, not at completely eradicating YCA for good, which is the project's eventual goal. If we left the island in its current state, YCA numbers would undoubtedly continue to increase, the infestation would return to the state it was found in in 2010, and all CAST efforts, expensive as they are, would be wasted. It is of the utmost importance to the success of the project that we come up with a baiting methodology that effectively kills YCA – queens

and all. For us, that means a lot of bioassay experiments and field trials, bait mixing and field site setup, ant counting and sunrises.

The baiting methodology that CAST III found so successful is a cat food based mixture. This bait mixture has the appearance of a delicious milkshake, and, to the ants, it has all the ingredients of one: cat food, pureed in water, fulfills all their protein-based needs and karo syrup satisfies their killer sweet tooth. Xanthan gum thickens the mixture, allowing the ingredients to go further and resulting in a consistency perfect for squirting out of super soakers, our preferred deployment method. The hidden addition of Safari (toxin = dinotefuran) kills the ants who consume it within a matter of days (maybe even hours).

When CAST IX arrived on Johnston, this was still the preferred baiting method. A few different toxins, all specific to invertebrates, were tried out over the years – Provant, Thiamethoxam, Tango – some with the same lethal effects, others with the intent of disrupting the reproductive viability of entire colonies by inhibiting larval growth, but none were as successful at reducing YCA numbers as Safari. We were sent out here with the task of doing a more thorough test of Thiamethoxam, and utilizing a different matrix that has worked well for Argentinian Ant eradication projects. This involves dissolving Thiamethoxam, which we lovingly refer to as T-Mex, in sugar-water and adding water-storing crystals (intended for garden and agricultural use) to soak up the resulting cocktail. The idea is that YCA will be attracted to the sugar in the crystals and suck up the moisture from them, poison and all. To test out this bait matrix, we've had to go through a number of steps – from testing its palatability to determining its effectiveness at killing YCA in the field.

CAST IX fooling around with some squirt guns which we use to apply cat food bait. Don't worry folks - the squirt guns in the photo had not yet come into contact with any poison!

The first question we needed to answer: Does this bait even attract YCA? If the YCA aren't biting, then the poison isn't getting to them, whether or not it would kill them if it did. For this, we conducted palatability trials, testing out crystals soaked in different concentrations of T-Mex. We set up card arrays containing 5 cards each – on 4 cards we placed crystals soaked in sugar-water containing 4 different T-Mex concentrations and on the fifth we placed crystals soaked just in sugar-water, as a control. We counted the number of YCA on each card at varying time intervals after deploying the bait – 10, 30, 60, and 90 minutes. In addition, we conducted time trials, to determine how long the average individual fed on each crystal concentration. For this, we timed feeding bouts from when an individual ant first made contact with a bait crystal until they left the card. Results showed that YCA were slightly more interested in bait crystals containing less T-Mex, but the difference was not too great to rule out the crystals containing higher concentrations as effective YCA attractants. We decided to move forward in testing the ant killing abilities

of all four concentrations of T-Mex in the Ant Lab.

YCA feeding on bait crystals during palatability trials. Photo courtesy of Kevin Donmoyer.

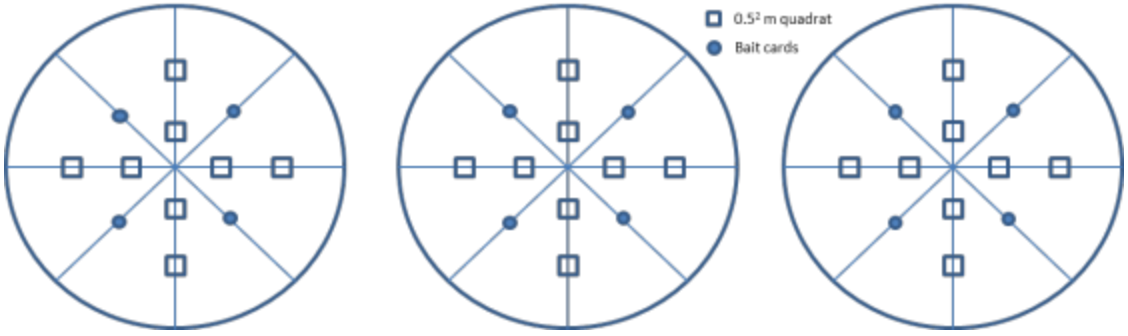
The Ant Lab is a bunker within the infestation area where we rear captive YCA so that we can conduct experiments and observations on them. We have several nest boxes filled with fully-functioning colonies that we feed and otherwise take care of… until we kill them with poison (or attempt to, anyway). We stock these colonies with queens and workers that we collect in the field, through a process that we refer to as “queening.” Basically, this involves going to a YCA-dense area, sifting through leaf-litter and dirt at the base of trees, and hoping to stumble upon a nest. It’s a very hit-or-miss process – sometimes we find enormous nests with hundreds of queens almost right away and other times it takes us hours to collect the number of queens and workers that we’re aiming for. But, when we do hit a nest, there’s no mistaking it. Worker ants pour out of their nests, often carrying eggs and larvae, and within seconds, we’re covered in them from head to toe, breathing in formic acid. This allows for very convenient worker collection – all we have to do is stick our hands in the outpour, let them climb right up, and shake them off into a bin that we use

to transfer them back to the lab. Spotting and collecting the queens is not so easy. Sometimes, even if a nest contains a lot of eggs and larvae, the queens may be just out of reach, hiding under a root or slab of concrete that we just can't get under. When exposed, they are expert hiders and make such a speedy run for cover that we need to work incredibly fast to collect them before they disappear once again. This often creates a situation in which we're all sitting under a tree, with our faces covered in ants, yelling "Queen!" back and forth so that someone else can get a queening bin ready. By the time we're done collecting, we're so covered in ants that we have to jump into the ocean just to get them off and ensure that we aren't transporting them to any of the non-YCA-infested areas of the island. Luckily, I think most of us have at least gotten used to being mobbed by YCA as part of the job. Some of us might even kind of enjoy it…

After a successful queening session, we brought eight colonies worth of ants (5 queens and ~100 workers per colony) back to the lab and set them up in cozy nest boxes. We provided them with fresh water, but deprived them of sugar-water and protein for two days so they'd be extra hungry when we exposed them to a sugary, poison-filled snack. Five of the colonies were given a few bait crystals soaked in one of the four T-Mex concentrations or a Safari-laced alternative. Three lucky colonies were given crystals soaked in just plain sugar-water, to be used as controls. We repeated this process a few times, each time with newly collected ants from the field, to ensure that our results were not just a fluke. For seven consecutive days after exposure, we checked on each colony, counting the number of live and dead workers and queens. Unfortunately, results were varied and not particularly clear. Within a couple of days, all the workers in colonies exposed to Safari were dead. Often a few queens would follow, without workers to attend to them. The colonies exposed to T-Mex, however, didn't appear to be very different from the controls. At least a few workers in each colony died and sometimes one or two queens were also found dead, but YCA's tendency to pile their dead and the erratic movement of the living made counting both dead and alive accurately very difficult, so it was hard to say whether one concentration was killing YCA more effectively than any other. We decided to move onto field trials, with the hope of clearer results.

We started by setting up six small-scale field sites that we refer to as tree arrays. Around a central, YCA infested tree, eight quadrats (0.5x0.5 meter squares outlined with flagging tape) and four bait tiles were staggered for the purpose of surveying ant population trends at each tree array, before and after bait applications (please see diagram below). For the quadrats, we simply counted the number of ants within the outlined square over the

course of 30 seconds. The tiles were to be used in the same way we use them on our regular surveys across the island – by placing a pea-sized dollop of pureed Spam in the center of the tile and returning an hour later to count the number of YCA attracted to it. After conducting a pre-monitor to get a baseline estimate of YCA numbers, we baited four tree arrays with varying concentrations of T-Mex, one with Safari, and left the last as a control (ideally the control would be baited with sugar-water crystals, but a limited quantity of necessary supplies meant we had to be as frugal as possible). For each baiting, we spread ~5L of poison-laced crystals evenly across the tree array's entire area. As enjoyable as squirting a cat food milkshake out of a super-soaker sounds, we quickly found that spreading bait crystals is way more fun. And efficient. The bait crystals bounce, spreading themselves, and fall into nooks and crannies that would otherwise be hard to reach with a squirt gun. More often than not, it's these hard to reach areas that are densest with YCA, so it works out pretty well. After applying bait, we again conducted ant counts over the course of several days, to get an idea of how the poison was affecting YCA.



Example of the tree-array monitoring setup with dots representing tiles and squares representing 0.5m quadrats. Tiles are 3m from the center and quadrats are placed 2m and 5m from the center. The radius of the entire array is 12.6m. Image courtesy of Dr. Robert Peck, USGS.

After several field trials using tree arrays and similar, but larger-scale, 2500m^2 plots, we found that the highest concentration of T-Mex that we tested leads to about a 50% reduction in detectable YCA, while the others basically did nothing. Safari, not surprisingly, led to almost complete elimination of YCA on the surface, when deployed in crystal form. Cat food applications of Safari, as well as a third bait matrix of textured vegetable protein (TVP) granules soaked in Safari-laced sugar-water, were similarly effective. The suggested 24L volume of bait crystals for baiting a 2500m^2plot seemed to us to be a bit overkill, and supplies, particularly sugar and water, are precious, so we tried halving and

quartering the recipe. Results suggested that 24L, 12L, and 6L applications of bait crystals were equally effective at knocking back YCA numbers, so it remains unclear what volume, if any, will become the Johnston standard. In a few weeks, CAST X will arrive on Johnston, carrying two literal tons of sugar and copious amounts of fresh water, with the intention of completing several consecutive infestation-wide bait applications, alternating the use of bait crystals, cat food, and TVP to conceal Safari, in order to discourage bait-shyness in YCA. We have high hopes that hitting YCA repeatedly with lethal poison will eventually reach all those queens hidden underground – either through their own consumption of Safari or through starving them of worker-provided aid.

While conducting field trials, we placed Spam on the tiles before sunrise, as YCA are most active during the cooler parts of the day. The hour in between spamming and counting was often spent watching a spectacular sunrise, which always seemed to make waking up so early worth it. Photo courtesy of Alison Zukas.

Field biology requires a lot of steps, supplies, back-up plans, and patience. It can be frustrating and unpredictable and is never exact. But, more often than not, those who do it

find it fun and rewarding. After six months on Johnston and a series of experiments that seemed would never end, we feel one step closer to ridding the island of a highly threatening pest. On top of all we've learned, seen, and experienced during a life-changing 6 month adventure, we can walk away optimistic about the future of a small island in the middle of the Pacific that we will always call home.

After baiting the area in which the blinded bird from above was nesting, YCA numbers in the area dropped, the bird's eyes healed completely, and a week later an adorable, fluffy chick hatched!

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